

Received 05/21/2004 13:40 in 02:06 on line (5) for RH10056 printed 05/21/2004 13:44 * Pg 2/8
May-21-2004 01:50pm From T-082 P.002/008 F-815

PATENT
*Please attach***IN THE UNITED STATES PATENT AND TRADEMARK OFFICE***In re* Application of:

Robert Michael ROBERTS Ex
Jonathan Andrew GREEN and
Sancai XIE

Group Art Unit: 1643

Examiner: C. Chen

Atty. Dkt. No.: UVMO:003

Serial No.: 09/273,164

Filed: March 19, 1999

For: COMPOSITIONS AND METHODS FOR
EARLY PREGNANCY DIAGNOSIS

CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited
with the U.S. Postal Service as First Class Mail in an envelope
addressed to: Assistant Commissioner for Patents,
Washington, D.C. 20231, on the date below:

Date

Robert E. Hanson

DECLARATION OF JONATHAN A. GREEN UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, JONATHAN A. GREEN, HEREBY DECLARE AS FOLLOWS:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-referenced patent application.
2. I am currently employed by The University of Missouri as an Assistant Professor. I hold a Ph.D. in Biochemistry from the University of Missouri. I have been conducting research in the area of biochemistry and reproductive biology since 1991.

Received 05/21/2004 13:40 in 02:06 on line [5] for RH10056 printed 05/21/2004 13:44 * Pg 3/8
May-21-2004 01:50pm From- T-082 P.003/008 F-815

3. I previously supplied a Declaration in this patent application submitting data demonstrating the isolation and use of monoclonal antibodies that detect PAGs disclosed in the above-referenced patent application. The data presented at that time demonstrated that PAGs 4, 6, 7, 16 and 20 are absent about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals. I am submitting this Declaration to present further data obtained since the time of the studies described in my previous Declaration. This data demonstrates that, in addition to the PAGs listed above, PAG 17 and PAG 21 are also undetectable at about two months post-partum.

4. *Identification of PAGs bound by L4, A6 and J2 monoclonal antibodies.*

The isolation of monoclonal antibodies L4, A6, and J2 was as described in my previous Declaration. Further studies were carried out under my supervision to identify the PAGs detected by these antibodies as follows:

One mg of each purified mAb was first crosslinked to 2 mL of matrix in the ImmunoPure Protein A IgG Plus Orientation kit (Pierce Biotechnology, Inc. Rockford, IL, USA) by following the manufacturer's instructions. Cotyledonary extracts were collected from 18 cm and 40 cm crown-rump bovine fetuses, dialyzed against 2000 volumes of binding buffer and 100 mg of total protein from each extract was applied separately to each matrix. The columns were washed in binding buffer until the absorbance of the flow-through at 280 nm was at baseline. The bound protein was eluted from the column with 20mM sodium formate, pH 2.8. The eluted proteins were subjected to SDS-PAGE followed by in-gel trypsin digestion, reduction and alkylation of cysteines. The masses of the resulting peptides were then determined by Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a Voyager-DE™ PRO Biospectrometry Workstation (Applied Biosystems, Foster City, CA, USA). The monoisotopic masses in the acquired spectra were used for searching against a nonredundant translated mammalian sequence database (NCBI) by using the Protein Prospector MS FTT program (<http://prospector.ucsf.edu/>).

The A6 monoclonal antibody exhibited the greatest ability to bind PAG in the placental extracts. The eluted material migrated at three distinct relative molecular mobilities on SDS-PAGE: 55,000, 65,000 and 75,000. Peptide mass fingerprinting revealed that the 75kDa band

Received 05/21/2004 13:40 in 02:06 on line [5] for RH10056 printed 05/21/2004 13:44 * Pg 4/8
May-21-2004 01:50pm From- T-092 P.004/008 F-915

consisted predominantly of PAG7 and PAG6 and the 55kDa band consisted predominantly of PAG16 and PAG4, although weak matches were also observed for PAG17, PAG20 and PAG21 in this band (Table 1). The 65kDa band did not produce many peptides amenable to fingerprinting, but the few that were produced were found to match PAG7.

The yields from the J2 and L4 affinity columns were not as robust as those from the A6 matrix, however, both did permit the purification of a ~70,000 Da protein. The J2-purified protein did not produce many tryptic fragments, but those that were produced matched PAG20 (Table 2). The L4-purified protein was more easily digested and produced numerous masses for fingerprinting. The main PAG isolated from the extracts was PAG21 although other PAGs (PAG17, PAG16 and PAG20) were clearly represented, albeit at lower concentrations (Table 3). The A6 and L4 antibodies bound PAG17 with lower affinity, but the results confirmed the undetectability of this PAG at about two-months post-partum. The major PAGs recognized by each of the monoclonal antibodies (and their relationship to other bovine PAGs) are indicated in the neighbor-joining tree (FIG. 1).

5. The result of the studies demonstrated that PAGs 4, 6, 7, 16, 17, 20 and 21 are undetectable about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals.

6. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

21 May 2004

Date

Jonathan A. Green

Jonathan A. Green

Received 05/21/2004 13:40 in 02:06 on line [5] for RH10056 printed 05/21/2004 13:44 * Pg 5/8

May-21-2004 01:51pm From-

T-092 P.005/008 F-915

Table 1. Assignment of tryptic digest fragments of PA3 affinity purified with the Ag immunodendritic antibody.

75 kDa protein

1. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 7.	2. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 7.	3. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 7.	4. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 7.
Observed	Identified	Delta mass	Start
829.344	829.342	-0.002	356-358 (QVGLAR)
727.418	727.416	-0.002	250-252 (QVGLAR)
765.420	765.420	0.000	130-132 (QVGLAR)
871.490	871.491	0.001	334-336 (QVGLAR)
1040.5410	1040.531	-0.010	303-305 (QVGLAR)
1238.0130	1238.023	0.010	137-147 (QVGLAR)
1254.8170	1254.812	-0.005	137-147 (QVGLAR)
1479.8730	1479.784	-0.089	30-42 (QVGLAR)
1480.7300	1480.729	-0.001	253-255 (QVGLAR)
1621.0130	1621.799	0.786	310-322 (QVGLAR)
1670.8530	1670.817	-0.036	309-322 (QVGLAR)
2209.3360	2209.221	-0.115	218-242 (QVGLAR)
2540.1670	2540.172	0.005	218-242 (QVGLAR)
2895.3420	2895.322	-0.020	146-171 (QVGLAR)

55 kDa protein

1. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 16.	2. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 16.	3. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 16.	4. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 16.
Observed	Identified	Delta mass	Start
829.300	829.302	0.002	374-376 (QVGLAR)
727.418	727.416	-0.002	250-252 (QVGLAR)
1040.530	1040.531	0.001	303-305 (QVGLAR)
1167.591	1167.591	0.000	242-251 (QVGLAR)
1380.620	1380.620	0.000	218-228 (QVGLAR)
1402.6410	1402.641	0.000	142-144 (QVGLAR)
1422.6470	1422.647	0.000	335-344 (QVGLAR)
1430.6790	1430.728	0.049	115-123 (QVGLAR)
1440.6890	1440.728	0.039	115-123 (QVGLAR)
1670.8770	1670.866	-0.011	161-163 (QVGLAR)
1822.7790	1822.799	0.020	102-104 (QVGLAR)
1821.7670	1821.761	-0.006	102-104 (QVGLAR)
1950.9090	1950.908	-0.001	43-64 (QVGLAR)
1739.8900	1739.892	0.002	113-123 (QVGLAR)
1950.9220	1950.975	0.053	216-231 (QVGLAR)
2012.0530	2012.057	0.004	185-212 (QVGLAR)

2. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 2.

Observed	Identified	Delta mass	Start
829.344	829.342	-0.002	373-377 (QVGLAR)
833.444	833.447	0.003	353-358 (QVGLAR)
1311.530	1311.532	0.002	359-364 (QVGLAR)
1237.856	1237.871	0.015	128-135 (QVGLAR)
1375.636	1375.636	0.000	380-372 (QVGLAR)
1430.714	1430.714	0.000	114-125 (QVGLAR)
1780.809	1780.809	0.000	117-123 (QVGLAR)
1782.009	1782.009	0.000	117-123 (QVGLAR)
1771.697	1771.697	0.000	126-111 (QVGLAR)
1770.649	1770.639	-0.010	126-111 (QVGLAR)
2207.186	2207.186	0.000	194-211 (QVGLAR)
2300.273	2300.273	0.000	230-251 (QVGLAR)

2. 80S TRAPLUS (AF102009) pregnancy-associated glycoprotein 4.

Observed	Identified	Delta mass	Start
842.477	842.473	-0.004	352-357 (QVGLAR)
1040.5	1040.531	0.031	303-305 (QVGLAR)
1080.817	1080.817	0.000	183-185 (QVGLAR)
1244.612	1244.638	0.026	128-135 (QVGLAR)
1291.870	1291.791	-0.079	353-364 (QVGLAR)
1340.680	1340.742	0.062	322-334 (QVGLAR)
1400.490	1400.403	-0.087	115-123 (QVGLAR)
1622.850	1622.855	0.005	7-181 (QVGLAR)
1622.880	1622.855	-0.025	40-184 (QVGLAR)
1681.012	1681.025	0.013	319-324 (QVGLAR)
2012.081	2012.087	0.006	195-212 (QVGLAR)
2191.686	2191.686	0.000	345-361 (QVGLAR)
2200.211	2200.211	0.000	287-288 (QVGLAR)
2441.282	2441.282	0.000	195-212 (QVGLAR)
2547.232	2547.232	0.000	35-64 (QVGLAR)
2700.483	2700.483	0.000	172-198 (QVGLAR)
3000.682	3000.681	-0.001	220-317 (QVGLAR)

* Number matches were observed for PA3-17, PA3-20 and PA3-31

Table 2. Assignment of tryptic digest fragments of PA6 affinity purified with the J2 monoclonal antibody.

BOS TAURUS. (AF182337) pregnancy-associated glycoprotein-20					
m/z submitted	MH+ matched	Delta ppm	Start	Peptide Sequence	Modifications
1046.585	1046.5811	52.4526	382-389	(R)LYFSVFDR(G)	
1687.861	1687.8607	0.2052	29-42	(R)KTLSDGKNNLNFLK(E)	1PO4
1788.976	1788.9271	26.7724	123-138	(R)LTNKTFGITYGSGRMK(G)	1Met-ox
1784.862	1784.8039	43.5329	217-221	(K)GSYVMFSGVDHRYN(G)	1PO4
1886.032	1886.0506	-8.7585	3-18	(K)WLVLLGLVAFSECFK(I)	
1927.919	1927.9264	-3.8439	327-342	(R)AYVLKDFGNCYTTFK(E)	
2221.268	2221.1368	59.1792	117-138	(R)QSSTFRLTNKTFGITYGSGR(M)	

T-082 P.006/008 F-815

May-21-2004 01:51pm From-

Received 05/21/2004 13:40 in 02:06 on line [5] for RH10056 printed 05/21/2004 13:44 * Pg 6/8

Table 3. Assignment of tryptic digest fragments of PAG affinity purified with the L4 monoclonal antibody.

BOS TAURUS. (AF182358) pregnancy-associated glycoprotein-21.

<u>m/z submitted</u>	<u>M+1+ matched</u>	<u>Delta m/z</u>	<u>Start</u>	<u>Peptide Sequence</u>	<u>Modifications</u>
642.527	642.5464	12.828	282-288	(K)LVNKGCK(L)	
665.513	665.542	-30.0409	327-334	(R)AYILKDSR(G)	
670.616	670.6413	-23.0012	281-288	(R)KLVNKGCK(L)	
1032.578	1032.5155	60.5738	363-369	(R)VYFSVFDR(G)	
1086.596	1086.5376	59.4428	127-136	(K)TFSITYGSGR(M)	
1176.673	1176.6646	7.1432	327-336	(R)AYILKDSRGK(C)	
1201.61	1201.6152	-4.3171	337-345	(R)CYTAKKKQR(F)	
1369.723	1369.6988	46.4078	215-228	(R)EGSYVMFGGVDHR(Y)	
1405.714	1405.6534	43.0848	218-228	(R)EGSYVMFGGVDHR(Y)	1Met-ox
1733.952	1733.8838	39.4834	113-128	(R)FRCHQSSTFRPTNK(T)	
1830.003	1829.9074	82.2487	346-361	(R)F88STETWLLGDAFLR(V)	
1860.005	1859.875	82.8738	215-231	(R)EGSYVMFGGVDHRYVK(G)	1Met-ox
1969.099	1968.9925	53.2688	232-248	(R)GELNWPVLEEGLWSVR(M)	
2153.122	2153.0327	41.4774	30-47	(K)TLSGKNMLNNFLKEHGK(L)	1PO4

Weaker matches were observed for PAG-17, PAG-18 and PAG-20

Received 05/21/2004 13:40 in 02:06 on line [5] for RH10056 printed 05/21/2004 13:44 * Pg 8/8
May-21-2004 01:51pm From- T-082 P.008/008 F-818

